3. Actomyosin is extractable from very fresh codling muscle at ionic strengths as low as 0·1 and pH 7·5, but quickly becomes insoluble. It is not extractable from fish under the same conditions after rigor mortis.

4. Monodisperse myosin is present in extracts of codling muscle made at an ionic strength of 0·3 or higher, pH 6·5 and 7·5.

5. Codling myosin is extremely labile and rapidly aggregates either on standing in the cold or during precipitation.

The assistance of Mr P. F. Howgate in the conduct of the experiments described in this paper is acknowledged. The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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The Charged Groups at the Interface of Some Blood Cells

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(*Received* 8 *October* 1957)

It has been implied that, apart from their quantitative differences in charge density, the ionized groups comprising the electrophoretic surface of foreign particles or cells suspended in plasma are similar and are probably derived from adsorbed protein. This conception is unsatisfactory in that it fails to explain the quite different colloidal properties of the formed elements of blood suspended in their common protein pool at a common bulk pH. Abramson, Moyer & Gorin (1942) emphasize the unusual fact that erythrocytes do not appear to adsorb protein, a property which, from the biological point of view, may help to explain many of their unusual properties. Thus erythrocytes do not ordinarily stick to one another, they do not adhere like leucocytes to glass, they survive for many weeks in a circulation constantly and efficiently being filtered of most other particles by reticulo-endothelial cells, and although they represent a huge surface area they do not, apparently, accelerate the clotting reaction in shed blood. With these simple biological properties in mind, it was thought worth while to investigate the nature of the ionized groups responsible for the surface charge of the various blood cells and particles added to blood, and perhaps to correlate any differences or similarities with their known biological properties. It was thought that lymphocytes, for instance, since they are not phagocytic, do not aggregate with polymorphonuclear leucocytes (Jago, 1956) and do not stick to glass, might have ionized groups similar to those found on an erythrocyte. On the other hand, it was thought probable that polymorphonuclear leucocytes, platelets and glass

particles exposed to plasma might have ion groups in common with one another; either these groups are capable of cation-bridging phenomena, or cell adhesion might take place by the attaction of mosaics of plus and minus charges of suitable stereochemical configuration.

No complete study of the comparative colloid properties of the circulating blood cells has been made in the past. The erythrocyte has received most attention, and amongst the many methods aimed at analysing its surface properties are those of electrophoresis (Abramson, 1930; Furchgott & Ponder, 1941), electrophoretic-charge reversal by various cations (Bungenberg de Jong, 1940–41; Seaman & Pethica, 1957), the action of detergents (Pethica & Schulman, 1953) and analysis of stripped-membrane components (Lovelock, 1955). Most authorities agree that the major functional components are phospholipid in nature but that protein and cholesterol may also be present near the surface. Furchgott & Ponder (1941) go further, and from their electrophoretic pH–mobility studies suggest that the erythrocyte surface is dominated by strongly acidic groups such as those of a phosphoric acid.

The electrophoretic pH–mobility curves for glass, quartz, collodion and mineral oils exposed to plasma suggested to Abramson (1929) the adsorption of protein. Polymorphonuclear leucocytes, lymphocytes and platelets have been less well studied and apart from isolated ζ potential estimations (Abramson, 1928; Fritze, 1953) and the classical miscibility experiments of Mudd & Mudd (1931) little is known of the surface components of these cells.

This paper makes use of the principle (Kruyt, 1948) that the ion groups (e.g. amino, phosphate, carboxyl or sulphate) contributing to the electrokinetic potential at a solid–liquid interface can be characterized by investigating the binding characteristics of a series of cations. The cations studied may be arranged in their order of effectiveness in reversing the charge of the particles as measured in a micro-electrophoresis cell. This order of effectiveness is referred to below as a 'charge-reversal spectrum'. The spectra obtained for various living cells may then be compared with spectra obtained from model systems with known charge groups at the surface. Information of this type has been published for the pig erythrocyte (Bungenberg de Jong, 1940–41) and *Escherichia coli* (Davies, Haydon & Rideal, 1956).

METHODS

Preparation of blood-cell suspensions. The various cells studied in the present investigation were obtained from a number of sheep. Whereas the platelets, erythrocytes and plasma for 'coating' the glass particles were those of one animal, the polymorphonuclear leucocytes and lymphocytes by necessity were obtained from different animals. To prepare platelets and erythrocytes, 9 vol. of venous blood was withdrawn carefully from the jugular vein into centrifuge tubes treated with silicone and containing 1 vol. of 3·8% (w/v) sodium citrate. The blood was centrifuged at 3000 rev./min. for 90 sec. and the platelet-rich plasma pipetted into 10 vol. of 0·145M-NaCl soln. The platelets were spun off and washed twice with 100 vol. of saline. The erythrocytes were likewise washed twice with 100 vol. of 0·145M-NaCl soln. and used from a stock suspension of approx. 1% (v/v).

Lymphocytes were obtained by slicing fresh mesenteric lymph glands from recently slaughtered animals and gently shaking the slices in 0·145M-NaCl soln. The lymphocytes, which fall out quite easily, were then washed twice with 100 vol. of saline and finally made up as a stock suspension of approx. 2% (v/v). Lymphocytes obtained from these lymph glands were observed microscopically to be a heterogeneous collection consisting of small, intermediate and large cells (a finding which might explain the rather large scatter of electrophoretic mobilities obtained for the lymphocyte suspensions at lower pH values).

Polymorphonuclear leucocytes were harvested by peritoneal lavage after a provocative injection of some 500–1000 ml. of 0·145M-NaCl soln. The peritoneal exudates were collected in tubes treated with silicone and containing heparin so as to give a final concentration of about 5 units/ml. The cellular deposit, after light centrifuging and washing, was checked for the presence of polymorphonuclear leucocytes by preparing a dry film and lightly staining with Leishman's stain. A stock suspension corresponding to approx. 2% (v/v) was used.

Plasma-coated glass particles. The glass particles were obtained by grinding Hysil glass to the consistency of fine sand, differentially centrifuging in water to obtain particles of approx. 10μ in size and finally washing with chromic acid. After thorough rinsing with water and saline, approx. 1 vol. of glass particles was mixed with 9 vol. of centrifuged (2500 *g* for 30 min.)., heparinized (10 units/ml.) sheep plasma. After contact for 5 min. the glass particles were centrifuged off and thrice washed with 100 vol. of 0·145N-NaCl and finally made up to a stock suspension of approx. 1% (v/v).

Model of substances. The specimens of 'egg lecithin' (lecithin–kephalin, 4:1) and phosphatidylethanolamine were kindly supplied by Dr D. N. Rhodes and conformed to the following limits of purity. The samples were fatty acid-free and contained less than 0·5% of free amino acid nitrogen (Lea, Rhodes & Stoll, 1955; Lea, 1953). Dr R. M. C. Dawson kindly supplied a chromatographically pure sample of phosphatidylcholine. The phosphatides were dispersed in 0·145M-NaCl soln. by shaking vigorously in a stoppered Pyrex tube.

Electrophoretic mobilities. Mobilities were measured by direct microscopic observation of the moving particles in a horizontal electrophoresis cell mounted in a water bath at 25° (Alexander & Saggers, 1948). Observations were made through an optically flat face ground in the wall of a 2 mm. diam. precision-bore capillary tube. The microscope assembly consisted of a 1·7 mm. objective with a ×10 graticule eyepiece giving a magnification ×200. Henry's optical correction for cylindrical cells was applied (Henry, 1938). A potential of $50 \pm 0{\cdot}2$ v was applied between two grey platinum electrodes 18 cm. apart at the ends of the capillary. Comparison of the potential gradient calculated

from the total current and the specific resistance of the electrolyte, with the potential gradient calculated from the applied potential and the tube length, showed good agreement, confirming that no serious polarization occurred at the electrodes. A Pye pH meter (Model 605, accuracy 0·02 unit) was used throughout.

In the suspension populations the maximum observed variation in mobility in each experiment was about 10% except with lymphocytes at low pH. Not less than five cells or particles were examined in each experiment.

All electrophoretic measurements were made on samples from freshly prepared stock suspensions (up to 12 hr. old). The 0·01% suspensions used in the actual mobility estimations were prepared by diluting the stock with 0·145M-NaCl soln. 1–2 min. before the run. In low and high pH runs (below 4 or over 9) the mobilities were estimated within 60 sec. of diluting the stock suspensions. If a change in mobility of below 20% occurred during a given set of observations, due possibly to cell breakdown, the observations were rejected. The results refer to a system in equilibrium with atmospheric CO_2 and O_2. The mobility of the suspension usually remained constant for at least 30 min. provided that the system was not below about pH 4.

The metal salts were used as solutions of the chlorides, with the exception of $Th(NO_3)_4$ and $UO_2(NO_3)_2$. The metal salts were A.R. grade, except the $LaCl_3$, and were made up in 0·145M-NaCl soln. and filtered.

Since it is difficult to adjust a wide range of metal-salt solutions to neutral pH without causing precipitation and complex changes (Brosset, Biedermann & Sillen, 1954; Ahrland, 1949; Hietanen, 1954), the solutions were used unbuffered. The natural pH values of the salt solutions were largely unaffected by the addition of the cells or particles (shifts less than 0·2 pH unit). All the electrolytes gave values in the range pH 4·5–6·0, except $UO_2(NO_3)_2$, which at a concentration of 0·1N has a pH of 3.

The water used in these experiments was obtained freshly distilled from a Pyrex still. All glassware was cleaned with chromic acid–sulphuric acid and rinsed repeatedly with water.

The mobilities ($\bar{V}$) of the cells and the particles were calculated in $\mu sec.^{-1} v^{-1} cm.^{-1}$ and were converted into ζ potentials by use of the Helmholtz–Smoluchowski equation $\zeta = (4\pi\eta/D)\,\bar{V}$, where η and D are the viscosity and the dielectric constants of the suspending medium. The use of surface and ζ potentials to calculate surface (or intrinsic) pK values for acidic and basic groups located in interfaces has been discussed elsewhere (Davies, Haydon & Rideal, 1956; Betts & Pethica, 1956; Seaman & Pethica, 1957).

RESULTS

The pH–mobility curves for sheep erythrocytes, polymorphonuclear leucocytes, lymphocytes and platelets are set out in Figs. 1 and 2, and for the phosphatide models, clean and plasma-coated glass in Figs. 4 and 5. The charge-reversal spectra are set out in Figs. 3 and 6.

In these spectra the reversal-of-charge concentration (C) represents the concentration (in normality) of the specified electrolyte added. C is the sum of the free electrolyte in solution (C_f) plus the bound electrolyte (C_b) in equilibrium with C_f (i.e. $C = C_b + C_f$). At a fixed colloid concentration it has been shown that the values of C_b for various cations at their reversal-of-charge concentration are equal (Bungenberg de Jong, Booij & Wakkie, 1936). From this it follows that charge-reversal spectra may be given in terms of C as well as C_f. The relative positions of the ions in a spectrum for C should not be altered by the total colloid concentration, provided that it is kept constant for a given spectrum.

DISCUSSION

The electrophoretic method will give information only about those membrane components of the various cells which are in the electrophoretic charge-determining region. The experiments reported here were carried out in 0·145M-sodium chloride solution. This implies that the mean thickness of the ionic double layer in the aqueous extracellular phase will be 8 Å (Verwey & Overbeek, 1948). If one assumes that the boundary of shear is very close to the membrane surface, only those charged groups within ~ 10 Å of the surface may be expected to influence the electrophoretic properties of the cell. If the cell is serrated, the principal charge effects will be due to the outermost charged groupings. The effects of the various ions on the electrophoretic charge of the cells may be due in part to changes of surface shape (e.g. increase in crenation) and also to changes in the double-layer thickness. This second effect is of importance only when the experimental concentration of added electrolyte approaches or exceeds that of sodium chloride. For example, with 0·05M-cupric chloride in 0·145M-sodium chloride the effective double-layer thickness is reduced to 6 Å. The effect of Cu^{2+} ions in these concentrations may thus be due partly to rendering inner groups less effective in determining the electrophoretic charge. This effect will not apply to any of the more effective charge-reversing cations for any given cell. Nor will this effect be operative in the experiments on the effects of pH on mobility in which the concentration of uni-univalent electrolyte was maintained at 0·145M throughout. From these considerations the principal electrophoretic effects of the various cations may be ascribed to the specific binding of the cations to the charge-determining groups with a resultant decrease in net charge. The charge-reversal spectra will be a function of the exterior charged groups, and by comparing these spectra with those for model systems the nature of the charged groups on the membranes may be elucidated (Kruyt, 1948). It may be noted that the model systems should be very well characterized for significant results to be obtained. Small amounts of impurities may dominate the electrophoretic pattern of the model. For example, if a

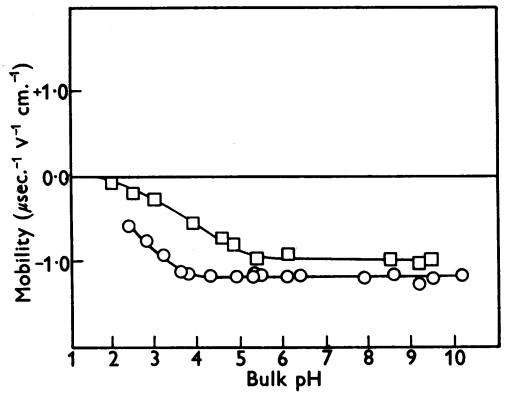

Fig. 1. pH–mobility relationships for sheep blood cells. □, Polymorphonuclear leucocytes; ○, erythrocytes.

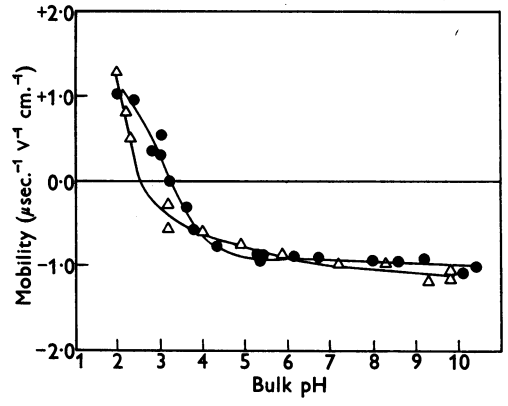

Fig. 2. pH–mobility relationships for sheep blood cells. △, Lymphocytes; ●, platelets.

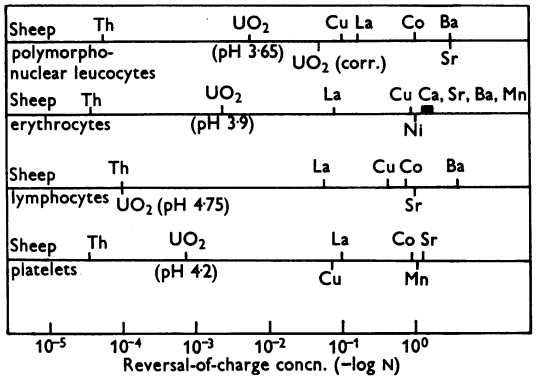

Fig. 3. Cation charge-reversal spectra for sheep blood cells.

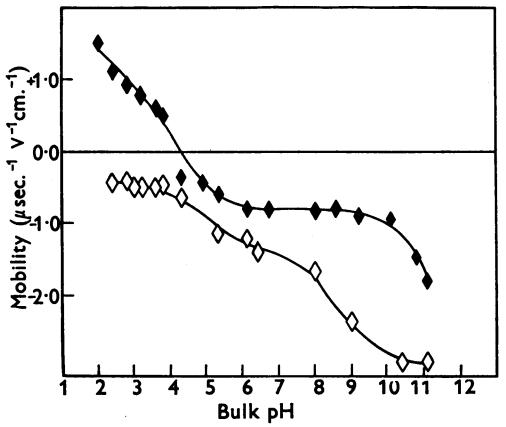

Fig. 4. pH–mobility relationships for acid-washed and plasma-coated glass. ◇, Acid-washed glass; ◆, plasma-coated glass.

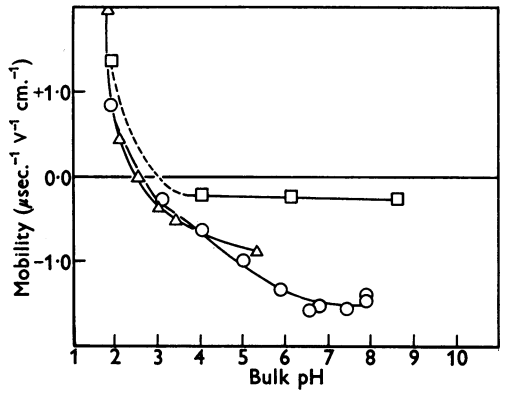

Fig. 5. pH–mobility relationships for phosphatides [concn. 0·01% (w/w) in 0·145M-NaCl]. □, Phosphatidylcholine; △, egg lecithin; ○, phosphatidylethanolamine.

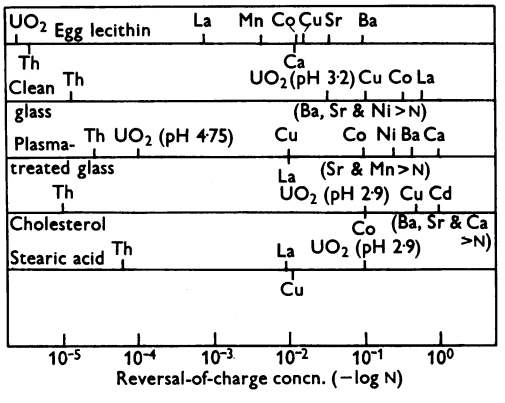

Fig. 6. Cation charge-reversal spectra for model systems.

phosphatide is dispersed to a particle size of 4 μ diameter, the surface molecules represent about 0·5 % of the total mass of each particle. If the dispersion contains an amount of impurity of this order the electrophoretic properties may be determined by the impurity if it is to be found preferentially in the surface. It is not likely that such small amounts of impurity would dominate the system in this way if the dispersed material is a true solid because of slow diffusion to the interface, unless there is a very wide difference in surface activity between the impurity and the main component. Thus the electrophoretic method offers a useful approach to the study of the exterior charged groups of living cells. The method may be expected to yield information on the adhesion properties of various cells on the basis of theories of colloidal stability in which the electrical double layer plays an important part (Verwey & Overbeek, 1948). The results for the various cells are discussed in this light.

The sheep polymorphonuclear leucocyte and erythrocyte exhibit similarities in the effect of pH on their electrophoretic mobilities (see Fig. 1). Neither cell exhibits a true isoelectric point since positive mobilities were not observed even when the pH was lowered to pH 1·8. This finding confirms the observations of Furchgott & Ponder (1941) for the erythrocyte. The erythrocyte haemolyses rapidly at low pH and apparent reversal of charge may be observed if haemolysate proteins were adsorbed by the cell membrane. Bungenberg de Jong (1940–41) attributes positive mobilities found in some cases to damage to erythrocytes. In the present experiments readings could be taken after as little as 45 sec. from making up the suspensions. Furthermore, it was found that erythrocytes exposed to low pH for periods similar to those required for readings to be taken exhibited normal electrophoresis on being returned to pH 5–6.

Apart from the joint lack of a true isoelectric point the polymorphonuclear leucocyte and erythrocyte are dissimilar electrophoretically. The effect of pH on the polymorphonuclear leucocyte mobility (Fig. 1) shows the presence of a singly-charged acidic group with pK 3·7. The almost constant mobility from pH 5·5 to 10 and the lack of charge reversal at low pH show clearly the absence of basic groups such as amines. The pK values for the polymorphonuclear leucocyte surface group strongly suggest that it is a carboxyl group. The pK values of monomeric carboxyl compounds likely to be found in biological systems range from 2·0 for the first pK of aspartic acid to 4·8 for short-chain fatty acids (Robinson & Stokes, 1955). Pectic acids, for example, are reported to have pK values of 2·95 (Davies *et al.* 1956) or 3·40 (Katchalsky, Shavit & Eisenberg, 1954). The last-named authors give the pK values for polyaspartic acid as 3·53 and the pK of polymethacrylic acid as 4·86. Electrophoresis of stearic acid particles give a pK of 4·2 on the assumption that the high ζ potential found can be treated by the Gouy theory (Verwey & Overbeek, 1948).

Comparison of the charge-reversal spectra for polymorphonuclear leucocytes with that for stearic acid or with the models used by Kruyt (1948) provides further evidence for the presence of carboxyl groups. The position, for example, of UO_2^{2+} ions (4×10^{-2} N, Fig. 6) is high, if allowance for the effect of pH of the uranyl nitrate solution at that concentration is made.

The electrophoretic mobility of the sheep erythrocyte, like that of the pig erythrocyte (Winkler & Bungenberg de Jong, 1940–41), varies with pH in a manner indicating the presence of one monobasic acid group in the surface. For sheep erythrocytes the pK of the surface group is 2·3. No amine groups are found on the surface since the electrophoretic mobility is constant from pH 4·5 to 11 and no charge reversal is found at low pH (Fig. 1). Furthermore, in the charge-reversal spectrum for erythrocytes (Fig. 3) it is found that UO_2^{2+} ions reverse the charge at a low concentration (2×10^{-3} N). A low charge-reversal concentration by UO_2^{2+} ions is characteristic of phosphatide and phosphate surfaces (Kruyt, 1948). The very low reversal-of-charge concentration for UO_2^{2+} ions acting on 'egg lecithin' solutions may be seen in Fig. 6. Uranyl ions bind very efficiently to synthetic lecithin monolayers (Anderson & Pethica, 1955), and also adsorb on alkyl phosphate monolayers (Parreira & Pethica, 1957). The erythrocyte surface pK 2·3 may thus be ascribed to a phosphate group. This value falls between the first pK of glycerol 2-phosphoric acid (1·33; Ashby, Crook & Datta, 1954) and that of mono-octadecyl phosphoric acid in the monolayer (3·0; Parreira & Pethica, 1957) and is very close to the first pK of orthophosphoric acid (2·14; Robinson & Stokes, 1955). This conclusion reinforces that of Furchgott & Ponder (1941).

Sheep platelets and lymphocytes, unlike the erythrocytes and polymorphonuclear leucocytes, exhibit true isoelectric points (as shown in Fig. 2) at pH 2·6 for lymphocytes and at pH 3·2 for platelets. This is clear evidence for the presence of positively-charged surface groups. The charge-reversal spectrum for lymphocytes (Fig. 3) is very similar to the cation spectrum for 'egg lecithin'. Similarly, the effect of pH on the mobility of 'egg lecithin' is almost identical with the effect of pH on lymphocyte mobility. The isoelectric point of 'egg lecithin' is 2·5, of phosphatidylethanolamine is 2·8 and of phosphatidylcholine 3·1. The strong

similarities in the effect of pH and in the charge-reversal spectra is very good evidence that the lymphocyte surface is a phosphatide, perhaps a kephalin. The charge-reversal spectrum for sheep platelets is again indicative of a phospholipid surface. Uranyl ion efficiently reverses the charge of sheep platelets, but not so efficiently as with 'egg lecithin' or lymphocytes. The similarity in the positions of lanthanum and copper in the spectrum, and the higher isoelectric point may indicate some carboxyl groups in the surface. It is hoped that it will be possible to obtain data on the electrophoretic properties of the carboxyl-containing phosphatidylserine in the near future. Both platelets and lymphocytes, as prepared in this work, accelerate the clotting of plasma in the Russel viper-venom test (A. D. Bangham, unpublished work). This is evidence for the presence of surface phosphatides, in view of the clotting acceleration found with phosphatide dispersions (P. Barkan & D. N. Rhodes, personal communication; O'Brien, 1956).

In view of the importance of glass surfaces in haematology, electrophoretic experiments on clean and plasma-treated glass particles were carried out (see Figs. 4 and 6). The effect of the adsorption of plasma components on the surface properties of glass may be clearly seen in these figures. The plasma-treated glass shows the presence of amphoteric groups with an isoelectric point of 4·2. The untreated glass surface is purely acidic. The sharp rise in negative mobility of plasma-treated glass above pH 9 is probably due to the desorption of the plasma components. The charge-reversal spectrum for plasma-treated glass is similar in many respects to that for platelets, as may be seen from the positions of lanthanum, copper and UO_2^{2+} ions. Differences arise in the position of strontium and the fact that manganese has almost no effect on plasma-treated glass. The effect of UO_2^{2+} ions and the pK of 4·2 suggests that the glass adsorbs a β-lipoprotein containing phospholipid. This conclusion could explain the well-known fact that glass surfaces initiate the clotting reaction in plasma.

These results, although obtained *in vitro* and in a saline medium, permit tentative conclusions to be drawn about the properties of the surface charge of sheep blood cells which are of value in the interpretation of a number of biological properties of the cells. Among these properties are the phagocytic power and 'stickiness' of the cells. Further work on the protein-adsorbing properties of the various cells will be necessary for fuller accounts of the properties *in vivo*. From published work on monolayer interactions (Doty & Schulman, 1949; Matalon & Schulman, 1949; Few, 1955), and on adsorption on stabilized oil emulsions (Fraser, 1957; Fraser & Schulman, 1956), there is good evidence that phosphatides adsorb a number of proteins only weakly in the physiological pH range. The surfaces of the platelet and lymphocyte will probably therefore largely retain their integrity in plasma. The erythrocyte is known to adsorb non-specific proteins very poorly, and in the circulation the surface-charge groups may be taken as similar to those found *in vitro* in saline. The importance of these findings for an interpretation of phagocytic activity is that the normal lymphocyte, platelet and erythrocyte escape phagocytosis more or less efficiently—the erythrocyte survives an efficient phagocytic filtration for 120 days. Changes on the erythrocyte surface, such as those due to antibody adsorption, do, however, permit sticking and phagocytosis. The blood cells are all negatively charged at physiological pH, and are, on considerations of overall charge alone, able to remain dispersed. Two mechanisms for sticking seem likely: (*a*) Direct sticking or aggregation due to the existence of a mosaic of plus and minus charges on a surface which is negative overall. Carboxyl and amine groups can be involved in binding of this type (Hill, 1956). (*b*) Sticking due to electrostatic bridging by bi- or multi-valent ions with or without the mediation of proteins. It is significant that the univalent carboxyl group is most evident only on those surfaces, i.e. polymorphonuclear leucocyte, platelet and plasma-coated glass, which show colloidal instability in the presence of plasma.

The sticking due to type (*a*) is unlikely for polymorphonuclear leucocytes and erythrocytes one to another, but is possible for platelets and lymphocytes. Sticking of type (*b*) would seem the more important. Platelets, for example, adhere more effectively to each other in the presence of Ca^{2+} ions. Phagocytosis by polymorphonuclear leucocytes is also dependent on the presence of Ca^{2+} ions (Mudd, McCutcheon & Lucke, 1934). Intra- and inter-molecular ionic bridging of proteins by cations is well known (Gurd, 1954). It is also possible that both oppositely and similarly charged groups can be involved in bridging owing to hydrogen bonding. The effects of hydrogen bonding of this kind in proteins have been discussed by Loeb & Sheraga (1956) and Laskowski & Sheraga (1954). Type (*b*) cross-linking of erythrocytes involving cations has been demonstrated by Jandl & Simmons (1957). The order of effectiveness in promoting agglutination of erythrocytes by a range of cations is different from the order of reversal of charge by cations. This itself is confirmation that the agglutination of erythrocytes by cations is due to direct cross-linking rather than to a simple reduction of charge or potential. It is noteworthy that Ca^{2+} ions are not efficient in agglutinating erythrocytes. This fact is in accord

with the finding of Clarke & Datta (1956) and Clarke, Cusworth & Datta (1954) that singly ionized glucose 1-phosphate interacts feebly with Ca^{2+} and Mg^{2+} ions, whereas the doubly ionized form interacts strongly with both Ca^{2+} and Mg^{2+} ions. This lack of reaction between singly ionized phosphate and Ca^{2+} ions is to be contrasted with the well-known binding of Ca^{2+} ions by fatty acids to form di-soaps. If it is postulated that erythrocytes are charged with phosphate groups, it appears that the lack of agglutination and protein binding, and escape from phagocytosis, is to be related to the properties of this group at interfaces. Preliminary results on the surface chemistry of phosphates have been published, and further publications will appear in due course (Parreira & Pethica, 1957).

SUMMARY

1. An attempt has been made to characterize the anion and cation groups contributing to the electrokinetic potential on the surface of certain blood cells, models and other particles.

2. The method involved the determination of the sequence or spectrum of various cations in their ability to reverse the charge of the cells and models in the presence of 0·145 M-sodium chloride solution. Actual mobilities expressed as $\mu sec.^{-1} v^{-1} cm.^{-1}$ were determined at values between pH 2 and 10.

3. In contrast with lymphocytes, phosphatide models and plasma-coated glass particles, neither the erythrocyte nor polymorphonuclear leucocyte exhibited a true isoelectric point.

4. The erythrocyte, however, differs markedly from the polymorphonuclear leucocyte with respect to the effect of UO_2^{2+} ions on its mobility. By comparison with known model systems it was concluded that the dominant group on the erythrocyte was a phosphate whereas that on the polymorphonuclear leucocyte was a carboxyl.

5. The cation spectra for lymphocytes and platelets are similar to one another and resemble that of a suspension of egg lecithin. It is suggested that the dominant groups are both phosphate and amine. Some carboxyl groups may also be present on the platelet. The surface of plasma-treated glass resembles the amine surface of a platelet in many respects.

6. Cation bridging between carboxyl surfaces is proposed as an important factor in the sticking and phagocytic reactions of blood cells.

Our thanks are due to Dr D. N. Rhodes and Dr R. M. C. Dawson for their generous gifts of phosphatide specimens, and to Dr J. H. Schulman and Sir Alan Drury, F.R.S., for stimulating discussions. One of us (G.V.F.S.) wishes to thank the Colonial Medical Research Council for a personal grant.

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Fungal Detoxication

3. THE METABOLISM OF ω-(2-NAPHTHYLOXY)-*n*-ALKYLCARBOXYLIC ACIDS BY *SCLEROTINIA LAXA**

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(*Received* 20 *August* 1957)

The metabolism of ω-(2-naphthyloxy)-*n*-alkylcarboxylic acids by *Aspergillus niger* van Tiegh. has been described in an earlier paper in this series (Byrde, Harris & Woodcock, 1956). By means of a replacement-culture technique it was shown that the acids were hydroxylated in position 6 of the naphthalene nucleus, and that higher members underwent β-oxidation.

The present paper describes an extension of the work to the plant pathogenic fungus *Sclerotinia laxa* Aderh. & Ruhl., the organism causing the brown-rot and blossom-wilt diseases of fruit trees.

EXPERIMENTAL

The materials and reference compounds have already been described (Byrde *et al.* 1956).

Methods

Replacement culture and isolation of metabolites. The method used followed that already described (Byrde *et al.* 1956), with the following modifications. Cultures of *S. laxa*, originally isolated from sour cherry, were grown on a liquid medium composed of: glucose, 40 g.; peptone, 10 g.; KH_2PO_4, 6·8 g.; $MgSO_4,7H_2O$, 2·5 g.; $CaCl_2$, 0·1 g.; $FeCl_3$, 0·02 g.; water to 1 l. The medium was dispensed in 50 ml. lots in 20 oz. medicinal 'flats' and sterilized (10 lb./sq.in. for 20 min.). Each bottle was inoculated with 6 disks (4 mm. in diameter) of mycelium cut from an established culture on solid medium, and incubated at 25° for 7 days. The medium was then replaced by 100 ml. of a solution of the test acid (0·5 mM) in 0·01 M-Na_2HPO_4. After a further 7 days the substrate was poured off, extracted with ether and examined chromatographically.

Fungitoxicity in vitro. Fungistatic activity was assessed by the rate of mycelial growth of *S. laxa* on an agar medium in which the compounds under test were incorporated. The liquid medium described above, with the addition of agar (3%, w/v), was dispensed in 50 ml. lots in 100 ml. flasks and autoclaved (10 lb./in.² for 20 min.). The pH was adjusted as necessary, after autoclaving, by the addition to each flask of sterile N-NaOH or N-H_2SO_4 by means of a sterile pipette; the amount required was estimated experimentally from a single flask.

The subsequent procedure followed that described by Bryde & Woodcock (1957).

RESULTS

Identification of metabolites. The metabolism of four unsubstituted and two hydroxylated ω-(2-naphthyloxy)-*n*-alkylcarboxylic acids was studied. Unchanged acid was detected in each instance. Table 1 summarizes the identification of the principal metabolites. In a typical experiment in which δ-(2-naphthyloxy)-*n*-valeric acid (220 mg.) in 0·01 M-Na_2HPO_4 (1800 ml.) was incubated with *S. laxa*, 46 mg. of β-(2-naphthyloxy)propionic acid, together with 45 mg. of unchanged acid, was isolated by large-scale paper chromatography as described previously (Byrde *et al.* 1956). Since β-naphthol was present in trace amounts only, after being washed with aqueous $NaHCO_3$ the ethereal extracts from a number of experiments were combined to obtain a specimen for mixed m.p. determination.

Fungitoxicity in vitro. The growth of *S. laxa* in glucose–peptone–phosphate agar in the presence of ethanol (1%, v/v) and a series of ω-(2-naphthyloxy)-*n*-alkylcarboxylic acids (0·25 mM) is shown in Table 2.

Since the pK_a value of 2-naphthyloxyacetic acid is 3·7 (Byrde *et al.* 1956), the more reliable comparison of fungistatic activity is that at pH 2·7, where the acids are predominantly undissociated (Simon & Blackman, 1949). The higher pH level

* Part 2: Byrde & Woodcock (1957).